

Claims:

1. A method for co-detecting Hepatitis C Virus (HCV) RNA and Human Immunodeficiency Virus (HIV) RNA in a biological sample, said method comprising:

(A) performing a reverse transcription reaction using RNA derived from said sample as a template and at least one reverse transcription primer that will prime reverse transcription of DNA from HCV RNA and at least one reverse transcription primer that will prime reverse transcription of DNA from HIV RNA to produce reverse transcription products comprising (a) HCV-specific reverse transcription products, (b) HIV-specific reverse transcription products, or (c) a combination of (a) and (b);

(B) amplifying said reverse-transcription products using one or more pairs of oligonucleotide primers specific for the 5' noncoding region of HCV and one or more pairs of oligonucleotide primers specific for HIV to produce amplification products comprising (a) HCV-specific amplification products, (b) HIV-specific amplification products, or (c) a combination of (a) and (b);

wherein each of said pairs of oligonucleotide primers specific for HCV comprises:

(i) forward primer 5'-
20 GGGAGAGCCATAGTGGTCTGCGGAA-3' (C131F25) <SEQ ID NO. 1>, and

(ii) reverse primer 5'-
CGGGGCACTCGCAAGCACCCATCA-3' (C294R25) <SEQ ID NO. 2>;

wherein each of the pairs of oligonucleotide primers specific for HIV-1 comprises a forward primer with the sequence:

5'-CTGCTTAAGCCTCAATAAGCTTGCCTTGA-3'
25 (JBLTR4) <SEQ ID NO. 3>, and a reverse primer specific for HIV-1 selected from the group consisting of:

(1) 5'-GGGTCTGAGGGATCTCTAGTTACC AGAGT-

3' (JBLTR6) <SEQ ID NO. 4>, and

(2) 5'-TGTTGGGCGCCACTGCTAGAGA-3'

(JBLTR8) <SEQ ID NO. 5>,

wherein each of the pairs of oligonucleotide primers specific for HIV-2 comprises a forward primer with the sequence 5'-GGGAGGTTCTCTCCAGCACTAGCA-3' (2LTRe) <SEQ ID NO. 6>, and a reverse primer specific for HIV-2 with the sequence 5'-GCGACTAGGAGAGATGGAACACACACA-3' (2LTR-R1) <SEQ ID NO. 7>; and

(C) detecting said amplification products;

wherein detection of HCV-specific amplification products indicates the presence of HCV RNA in said sample, detection of HIV-specific amplification products indicates the presence of HIV RNA in said sample, and the detection of HCV-specific amplification products and HIV-specific amplification products indicates the presence of HCV RNA and HIV RNA in said sample.

2. A method as defined in claim 1, wherein said reverse transcription reaction is performed using random oligonucleotide primers.

3. A method as defined in claim 1, wherein said reverse transcription reaction is performed using one or more oligonucleotide primers having sequences corresponding to sequences in HCV RNA and one or more oligonucleotide primers having sequences corresponding to sequences in HIV RNA.

4. A method as defined in claim 1, wherein said amplifying is performed by a method selected from the group consisting of polymerase chain reaction, ligase chain reaction, strand displacement amplification, nucleic acid single base amplification, and transcription mediated amplification.

5. A method as defined in claim 1, wherein said detecting comprises visualizing said amplification products by gel electrophoresis.

6. A method as defined in claim 1, wherein said detecting comprises capturing said amplification products on a solid support containing (a) one or more HCV-specific oligonucleotide probes, (b) one or more HIV-specific oligonucleotide probes, or (c) a combination of (a) and (b) and quantifying said captured products using a colorimetric assay.

7. A method as defined in claim 6, wherein said HCV-specific probe consists of 5'-CCTTCGCGACCAACACTACTCGGCT-3' (C252-27-PRB) <SEQ ID NO. 12> and said HIV-specific probe is selected from the group consisting of:

(a) 5'-CAACAGACGGGCACACACT-3' (JBLTRpr3)

<SEQ ID NO. 13>;

(b) 5'-GAACAGATGGGCACACACTGCT-3' (JBLTRpr4)

<SEQ ID NO. 16>; and

(c) 5'-CCACGCTTGCTTGCTTAAAGACCTC-3'

(2LTRpr1)

<SEQ ID NO. 14>.

8. A method as defined in claim 1, wherein said sample is selected from the group consisting of blood, serum, plasma, urine, saliva, and cerebrospinal fluid.

9. A method as defined in claim 1, wherein said co-detecting is simultaneous.

10✓ A method for co-amplifying Hepatitis C Virus (HCV) DNA and Human Immunodeficiency Virus (HIV) DNA, said method comprising:

(A) performing a polymerase chain reaction on a DNA sample suspected to contain HCV DNA, HIV DNA, or a combination of HCV DNA and HIV DNA, using one or more pairs of oligonucleotide primers specific for the 5' noncoding region of HCV and one or more pairs of oligonucleotide primers specific for HIV to produce amplification products comprising (a) HCV-specific amplification products, (b) HIV-specific amplification products, or (c) a combination of (a) and (b);

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10 wherein each of said pairs of oligonucleotide primers specific for HCV comprises:

(i) forward primer 5'-GGGAGAGCCATAGTGGTCTGCGGAA-3'

(C131F25) <SEQ ID NO. 1>, and

(ii) reverse primer 5'-CGGGCACTCGCAAGCACCCCTATCA-3'
(C294R25) <SEQ ID NO. 2>;

15 wherein each of the pairs of oligonucleotide primers specific for HIV-1 comprises a forward primer with the sequence:

5'-CTGCTTAAGCCTCAATAAAGCTTGCCTTGA-3'

(JBLTR4) <SEQ ID NO. 3>, and a reverse primer specific for HIV-1 selected from the group consisting of:

20 (1) 5'-GGGTCTGAGGGATCTCTAGTTACC AGAGT-

3' (JBLTR6) <SEQ ID NO. 4>, and

(2) 5'-TGTTCGGGCGCCACTGCTAGAGA-3'

(JBLTR8) <SEQ ID NO. 5>; and

25 wherein each of the pairs of oligonucleotide primers specific for HIV-2 comprises a forward primer with the sequence 5'-GGGAGGTTCTCTCCAGCACTAGCA-3' (2LTRe) <SEQ ID NO. 6>, and a reverse primer specific for HIV-2 with the sequence 5'-GCGACTAGGAGAGATGGAACACACA-3' (2LTR-R1) <SEQ ID NO. 7>.

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11. A method as defined in claim 10, further comprising:

(B) detecting said amplification products,

wherein detection of HCV-specific amplification products indicates the presence of HCV DNA in said sample, detection of HIV-specific amplification products indicates the presence of HIV DNA in said sample, and the detection of HCV-specific amplification products and HIV-specific amplification products indicates the presence of HCV DNA and HIV DNA in said sample.

12. A method as defined in claim 11, wherein said detecting comprises visualizing said amplification products by gel electrophoresis.

13. A method as defined in claim 11, wherein said detecting comprises capturing said amplification products on a solid support containing (a) one or more HCV-specific oligonucleotide probes, (b) one or more HIV-specific oligonucleotide probes, or (c) a combination of (a) and (b) and quantifying said captured products using a colorimetric assay.

14. A method as defined in claim 13, wherein said HCV-specific probe consists of 5'-CCTTCGCGACCCAACACTACTCGGCT-3' (C252-27-PRB) <SEQ ID NO. 12> and said HIV-specific probe is selected from the group consisting of:

(a) 5'-CAACAGACGGGCACACACT-3'

(JBLTRpr3) <SEQ ID NO. 13>;

(b) 5'-GAACAGATGGGCACACACTGCT-3'

(JBLTRpr4) <SEQ ID NO. 16>; and

(c) 5'-CCACGCTTGCTTGCTTAAAGACCTC-3'

(2LTRpr1) <SEQ ID NO. 14>.

15. A method as defined in claim 1, wherein said co-amplifying is simultaneous.

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16. A method for co-detecting Hepatitis C Virus (HCV) RNA and Human Immunodeficiency Virus (HIV) RNA in a biological sample, said method comprising:

(A) performing a reverse transcription reaction using RNA derived from said sample and internal positive control (IPC) RNA as a template, at least one reverse transcription primer that will prime reverse transcription of DNA from IPC RNA, at least one reverse transcription primer that will prime reverse transcription of DNA from HCV RNA, and at least one reverse transcription primer that will prime reverse transcription of DNA from HIV RNA to produce reverse transcription products comprising (a) IPC-specific reverse transcription products and (b) HCV-specific reverse transcription products, (c) HIV-specific reverse transcription products, or (d) any combination of any of the foregoing;

(B) amplifying said reverse-transcription products using one or more pairs of oligonucleotide primers specific for IPC, one or more pairs of oligonucleotide primers specific for the 5' noncoding region of HCV, and one or more pairs of oligonucleotide primers specific for HIV to produce amplification products comprising (a) IPC-specific amplification products (b) IPC-specific amplification products and HCV-specific amplification products, (c) IPC-specific amplification products and HIV-specific amplification products, or (d) a combination of any of the foregoing;

wherein each of said pairs of oligonucleotide primers specific for IPC

comprises:

(1) forward

primer

5'-

CGCCAGCGTGGACCATCAAGTAGTAA-3' (IPCF1) <SEQ ID NO. 8>, and

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5 (2) reverse primer 5'-
CACGATCCTGGAGCAGACACTGAAGA-3' (IPCR1) <SEQ ID NO. 9>;
wherein each of said pairs of oligonucleotide primers specific for
HCV comprises:

10 (i) forward primer 5'-
GGGAGAGCCATAGTGGTCTGCGGAA-3' (C131F25) <SEQ ID NO. 10>, and
(ii) reverse primer 5'-
CGGGGCACTCGCAAGCACCTATCA-3' (C294R25) <SEQ ID NO. 11>; and
wherein each of the pairs of oligonucleotide primers specific for
HIV-1 comprises a forward primer with the sequence:

15 5'-CTGCTTAAGCCTCAATAAAGCTTGCCTTGA-3'
(JBLTR4) <SEQ ID NO. 3>, and a reverse primer specific for HIV-1 selected from
the group consisting of:

20 (1) 5'-GGGTCTGAGGGATCTCTAGTTACC AGAGT-
3' (JBLTR6) <SEQ ID NO. 4>, and
(2) 5'-TGTCGGGCGCCACTGCTAGAGA-3'
(JBLTR8) <SEQ ID NO. 5>,

25 wherein each of the pairs of oligonucleotide primers specific
for HIV-2 comprises a forward primer with the sequence 5'-
GGGAGGTTCTCTCCAGCACTAGCA-3' (2LTRe) <SEQ ID NO. 6>, and a
reverse primer specific for HIV-2 with the sequence 5'-
GCGACTAGGAGAGATGGAACACACA-3' (2LTR-R1) <SEQ ID NO. 7>; and
(C) detecting said amplification products

30 wherein detection of IPC-specific amplification products
indicates the presence of IPC RNA in said sample, detection of HCV-specific
amplification products indicates the presence of HCV RNA in said sample, detection
of HIV-specific amplification products indicates the presence of HIV RNA in said
sample, and the detection of HCV-specific amplification products and HIV-specific

amplification products indicates the presence of HCV RNA and HIV RNA in said sample.

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17. A method as defined in claim 16, wherein said reverse transcription reaction is performed using random oligonucleotide primers.

18. A method as defined in claim 16, wherein said reverse transcription reaction is performed using one or more oligonucleotide primers having sequences corresponding to sequences in IPC RNA, one or more oligonucleotide primers having sequences corresponding to sequences in HCV RNA and one or more oligonucleotide primers having sequences corresponding to sequences in HIV RNA.

19. A method as defined in claim 16, wherein said amplifying is performed by a method selected from the group consisting of polymerase chain reaction, ligase chain reaction, strand displacement amplification, and transcription mediated amplification.

20. A method as defined in claim 16, wherein said detecting comprises visualizing said amplification products by gel electrophoresis.

21. A method as defined in claim 16, wherein said detecting comprises capturing said amplification products on a solid support containing (a) one or more IPC-specific oligonucleotide probes, (b) one or more HCV-specific oligonucleotide probes, (c) one or more HIV-specific oligonucleotide probes, or (d) a combination of any of (a), (b), and (c) and quantifying said captured products using a colorimetric assay.

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22. A method as defined in claim 21, wherein said IPC-specific probe consists of 5'-CTGCGTTAGACCGAGAACTGTGGATAAAGG-3' <SEQ ID NO. 17>, said HCV-specific probe consists of 5'-CCTTCGCGACCCAACACTACTCGGCT-3' (C252-27-PRB) <SEQ ID NO. 12> and said HIV-specific probe is selected from the group consisting of:

(a) 5'-CAACAGACGGGCACACACT-3'
(JBLTRpr3) <SEQ ID NO. 13>;
(b) 5'-AACAGATGGGCACACACTGCT-3'
(JBLTRpr4) <SEQ ID NO. 16>; and
(c) 5'-CCACGCTTGCTTGCTTAAAGACCTC-3'
(2LTRpr1) <SEQ ID NO. 14>.

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products, (b) IPC amplification products and HCV-specific amplification products, (c) IPC amplification products and HIV-specific amplification products, or (d) a combination of any of (a), (b), and (c);

wherein each of said pairs of oligonucleotide primers specific for IPC comprises:

(i) forward primer 5'-

CGCCAGCGTGGACCATCAAGTAGTAA-3' (IPCF1) <SEQ ID NO. 8>, and

(ii) reverse primer 5'-

CACGATCCTGGAGCAGACACTGAAGA-3' (IPCR1) <SEQ ID NO. 9>;

wherein each of said pairs of oligonucleotide primers specific

for HCV comprises:

(i) forward primer 5'-GGGAGAGCCATAGTGGTCTGCGGAA-3'

(C131F25) <SEQ ID NO. 10>, and

(ii) reverse primer 5'-CGGGGCACTCGCAAGCACCTATCA-3'

(C294R25) <SEQ ID NO. 11>; and

wherein each of the pairs of oligonucleotide primers specific for HIV-1 comprises a forward primer with the sequence:

5'-CTGCTTAAGCCTCAATAAGCTTGCCTTGA-3'

(JBLTR4) <SEQ ID NO. 3>, and a reverse primer specific for HIV-1 selected from the group consisting of:

(1) 5'-GGGTCTGAGGGATCTCTAGTTACC AGAGT-

3' (JBLTR6) <SEQ ID NO. 4>, and

(2) 5'-TGTTCGGGCGCCACTGCTAGAGA-3'

(JBLTR8) <SEQ ID NO. 5>,

wherein each of the pairs of oligonucleotide primers specific

for HIV-2 comprises a forward primer with the sequence 5'-
GGGAGGTTCTCTCCAGCACTAGCA-3' (2LTrE) <SEQ ID NO. 6>, and a

reverse primer specific for HIV-2 with the sequence 5'-
GCGACTAGGAGAGATGGAACACACA-3' (2LTR-R1) <SEQ ID NO. 7>.

26. A method as defined in claim 10, further comprising:

(B) detecting said amplification products,

wherein detection of IPC-specific amplification products indicates the presence of IPC DNA in said sample, detection of HCV-specific amplification products indicates the presence of HCV DNA in said sample, detection of HIV-specific amplification products indicates the presence of HIV DNA in said sample, and the detection of HCV-specific amplification products and HIV-specific amplification products indicates the presence of HCV DNA and HIV DNA in said sample.

27. A method as defined in claim 26, wherein said detecting comprises visualizing said amplification products by gel electrophoresis.

28. A method as defined in claim 26, wherein said detecting comprises capturing said amplification products on a solid support containing (a) one or more IPC-specific oligonucleotide probes, (b) one or more HCV-specific oligonucleotide probes, (c) one or more HIV-specific oligonucleotide probes, or (d) any combination of any of the foregoing and quantifying said captured products using a colorimetric assay.

29. A method as defined in claim 28, wherein said IPC-specific probe consists of 5'-CTGCGTTAGACCGAGAACTGTGGATAAAGG-3' <SEQ ID NO. 17>, said HCV-specific probe consists of 5'-CCTTCGCGACCCAACACTACTCGGCT-3' (C252-27-PRB) <SEQ ID NO. 12> and said HIV-specific probe is selected from the group consisting of:

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(a) 5'-CAACAGACGGGCACACACT-3'
(JBLTRpr3) <SEQ ID NO. 13>;
(b) 5'-AACAGATGGGCACACACTGCT-3'
(JBLTRpr4) <SEQ ID NO. 16>; and
(c) 5'-CCACGCTTGCTTGCTTAAAGACCTC-3'
(2LTRpr1) <SEQ ID NO. 14>.

30. A method as defined in claim 29, wherein said co-amplifying is simultaneous.

31. A kit for co-detecting HCV RNA and HIV RNA in a biological sample, said kit comprising:

(a) a pair of oligonucleotide primers specific for the 5' noncoding region of HCV comprising:

(i) forward primer 5'-
GGGAGAGCCATAGTGGTCTGCGGAA-3' (C131F25) <SEQ ID NO. 10>, and
(ii) reverse primer 5'-
CGGGGCACTCGCAAGCACCCTATCA-3' (C294R25) <SEQ ID NO. 11>; and

(b) oligonucleotide primers specific for HIV-1 which comprise a forward primer with the sequence:
5'-CTGCTTAAGCCTCAATAAGCTTGCCTTGA-3'
(JBLTR4) <SEQ ID NO. 3>, and a reverse primer specific for HIV-1 selected from the group consisting of:
(1) 5'-GGGTCTGAGGGATCTCTAGTTACC AGAGT-
3' (JBLTR6) <SEQ ID NO. 4>, and
(2) 5'-TGTCGGGCGCCACTGCTAGAGA-3'
(JBLTR8) <SEQ ID NO. 5>, and a pair of oligonucleotide primers specific for HIV-2 which comprise a forward primer with the sequence 5'-

GGGAGGTTCTCTCCAGCACTAGCA-3' (2LTRe) <SEQ ID NO. 6>, and a
reverse primer specific for HIV-2 with the sequence: 5'-
GCGACTAGGAGAGATGGAACACACACA-3' (2LTR-R1)
<SEQ ID NO. 7>.

32. A kit as defined in claim 31, further comprising a pair of
oligonucleotide primers specific for IPC, wherein said pair of oligonucleotide
primers specific for IPC comprises forward primer 5'-
CGCCAGCGTGGACCATCAAGTAGTAA-3' (IPCF1) <SEQ ID NO. 8> and
reverse primer 5'-CACGATCCTGGAGCAGACACTGAAGA-3' (IPCR1) <SEQ ID
NO. 9>.

33. A kit as defined in claim 31, further comprising one or more
probes.

34. A kit as defined in claim 32, further comprising one or more
probes.

35. A kit as defined in claim 33, wherein said probes are selected
from the group consisting of 5'-CCTTCGCGACCCAACACTACTCGGCT-3'
(C252-27-PRB) <SEQ ID NO. 12>, 5'-CAACAGACGGGCACACACTACT-3'
(JBLTRpr3) <SEQ ID NO. 13>, 5'-GAACAGATGGGCACACACTGCT-3' (JBLTRpr4)
<SEQ ID NO. 16>, and 5'-CCACGCTTGCTTAAAGACCTC-3' (2LTRpr1)
<SEQ ID NO. 14>.

36. A kit as defined in claim 34, wherein said IPC-specific probe
consists of 5'-CTGCGTTAGACCGAGAACTGTGGATAAAGG-3' (IPC1P) <SEQ

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ID NO. 17>, said HCV-specific probe consists of 5'-
CCTTCGCGACCCAACACTACTCGGCT-3' (C252-27-PRB) <SEQ ID NO. 12>,
and said HIV-specific probe is selected from the group consisting of:

- (a) 5'-CAACAGACGGGCACACACTACT-3'
(JBLTRpr3) <SEQ ID NO. 13>;
- (b) 5'-GAACAGATGGGCACACACTGCT-3'
(JBLTRpr4) <SEQ ID NO. 16>; and
- (c) 5'-CCACGCTTGCTTGCTTAAAGACCTC-3'
(2LTRpr1) <SEQ ID NO. 14>.

37. A kit for co-amplifying HCV DNA and HIV DNA in a DNA sample, said kit comprising:

- (a) a pair of oligonucleotide primers specific for the 5' noncoding region of HCV comprising:
 - (i) forward primer 5'-
GGGAGAGCCATAGTGGTCTGGAA-3' (C131F25) <SEQ ID NO. 10>, and
 - (ii) reverse primer 5'-
CGGGGCACTCGCAAGCACCCATCA-3' (C294R25) <SEQ ID NO. 11>;
- (b) oligonucleotide primers specific for HIV-1 which comprise a forward primer with the sequence:
5'-CTGCTTAAGCCTCAATAAGCTTGCCTTGA-3'

(JBLTR4) <SEQ ID NO. 3>, and a reverse primer specific for HIV-1 selected from the group consisting of:

- (1) 5'-GGGTCTGAGGGATCTCTAGTTACC AGAGT-3' (JBLTR6) <SEQ ID NO. 4>, and
- (2) 5'-TGTCGGGCGCCACTGCTAGAGA-3'
(JBLTR8) <SEQ ID NO. 5>, and a pair of oligonucleotide primers specific for HIV-2 which comprise a forward primer with the sequence 5'-

GGGAGGTTCTCTCCAGCACTAGCA-3' (2LTRe) <SEQ ID NO. 6>, and a
reverse primer specific for HIV-2 with the sequence: 5'-
GCGACTAGGAGAGATGGAACACACA-3' (2LTR-R1)
<SEQ ID NO. 7>.

38. A kit as defined in claim 37, further comprising a pair of oligonucleotide primers specific for IPC, wherein said pair of oligonucleotide primers specific for IPC comprises forward primer 5'-CGCCAGCGTGGACCATCAAGTAGTAA-3' (IPCF1) <SEQ ID NO. 8> and reverse primer 5'-CACGATCCTGGAGCAGACACTGAAGA-3' (IPCR1) <SEQ ID NO. 9>.

39. A kit as defined in ~~claim~~ 37, further comprising one or more probes.

40. A kit as defined in claim 38, further comprising one or more probes.

41. A kit as defined in claim 39, wherein said probes are selected
from the group consisting of 5'-CCTT~~T~~GGACCCAACACTACTCGGCT-3'
(C252-27-PRB) <SEQ ID NO. 12>, 5'-CAACAGACGGGCACACACTACT-3'
(JBLTRpr3)
<SEQ ID NO. 13>, 5'-GAACAGATGGGCACACACTGCT-3' (JBLTRpr4)
<SEQ ID NO. 16>, and 5'-CCACGCTTGCTTAAAGACCTC-3' (2LTRpr1)
<SEQ ID NO. 14>.

42. A kit as defined in claim 40, wherein said IPC-specific probe consists of 5'-CTGCGTTAGACCGAGAACTGTGGATAAAGG-3' (IPC1P) <SEQ

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ID NO. 17>, said HCV-specific probe consists of 5'-
CCTTTCGCGACCCAACACTACTCGGCT-3' (C252-27-PRB) <SEQ ID NO. 12>,
and said HIV-specific probe is selected from the group consisting of:

(a) 5'-CAACAGACGGCACACACT-3'

(JBLTRpr3)
<SEQ ID NO. 13>;

(b) 5'-GAACAGATGGCACACACTGCT-3'

(JBLTRpr4)
<SEQ ID NO. 16>; and

(c) 5'-CCACGCTTGCTTGCTTAAAGACCTC-3'

(2LTRpr1)
<SEQ ID NO. 14>.

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